1. THE MITOCHONDRION

Biochemists Confront Particulate Structure: Mitochondrial Enzyme Systems

During the same period in which the Rockefeller researchers were situating succinoxidase and other oxidative enzymes in the mitochondrion and Claude was identifying its role as the power plant of the cell, numerous biochemists were following up on the thread from Keilin and Hartree's (1944) demonstration that they could not eliminate cell particulates from extract preparations capable of performing oxidative phosphorylation. Keilin and Hartree (1949) themselves interpreted this as indicating that the respiratory function was connected with the physico-chemical structure of the cell.[3] For most biochemists, however, the involvement of cell particulates was, as Lehninger put it, "a nuisance" (1951).[4] Their goal was to work out the purely chemical steps in oxidative phosphorylation in a manner comparable to that already provided for glycolysis.

As discussed in Chapter 3, by the 1940s there was a good understanding of the major operations in the three metabolic mechanisms that worked in sequence to oxidize carbohydrates – glycolysis, the citric acid (Krebs) cycle, and the electron transport chain (see Figure 3.16). After the investigations of Kalckar (1939) and Lipmann (1939), it was recognized that oxidative metabolism was linked to the storage of energy via ATP formation. In a theoretical paper, Lippman (1941) characterized the phosphate bonds in ATP as *energy-rich bonds* and introduced the symbol ~P for these; later they were more commonly referred to as *high-energy* bonds. The process by which energy was stored in such bonds was understood earliest (and turned out to be simplest) for the first two mechanisms, glycolysis and the citric acid cycle. As detailed in Chapter 3, in glycolysis the initial substrate (glucose) and the penultimate product (pyruvic acid) do not contain phosphates, but the various intermediates do. Phosphates are added at three different steps in the process and in later steps are transferred to ADP, yielding energy-rich ATP. This process later became known as *substrate-level phosphorylation*.

Knowledge of the process by which phosphorylation was coupled to the third oxidative mechanism in the sequence, the electron transport chain, was

[3] Keilin and Hartree (1949) themselves proposed that the particulate nature of their preparation facilitated respiration by assuring the mutual accessibility of the different enzymes encapsulated within each particle. Cleland and Slater (1953) determined that the Keilin and Hartree preparation included membranes from mitochondria.

[4] See also Lehninger (1964): "It was a part of the *Zeitgeist* that particles were a nuisance and stood in the way of purification of the respiratory enzymes" (p. 6).